

THE EFFECTS OF BIOCHAR, WEED CONTROL, AND IRRIGATION
ON THE GROWTH AND SURVIVAL OF JACK PINE SEEDLINGS
AND NATIVE PLANT COMMUNITIES IN NORTHERN
MINNESOTA, USA

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Introduction

Climate change is predicted to result in more frequent and more severe droughts in the northern Midwest region of the United States (Janowiak et al. 2014; Millar et al. 2007; Weed et al. 2013; Vose et al. 2016). This has and will continue to impact forests of the region, especially in regards to establishing regeneration during periods of drought stress (Allen et al 2010; Janowiak et al 2014; Tardif and Conciatori 2001; Windmuller-Campione et al 2019). This is leading researchers and land managers to explore adaptive silviculture strategies to promote resilience in their forests (Nagel et al 2017; Millar et al 2007).

One option hypothesized to improve regeneration success in forest systems is the application of biochar to the soil to increase seedling resistance and resilience to drought conditions (Basso et al 2013; Karhu et al 2011; Spokas et al 2009). Biochar is a bio-based soil amendment created through pyrolysis that has been used for centuries in tropical environments to increase productivity (Sohi et al 2010; Lehmann and Joseph 2015; Lehmann et al 2003). Since then, biochar has been widely used in agriculture, as it has proven to be effective in increasing nutrient cycling, cation exchange capacity, and water holding capacity of the soil (Borchard et al 2014; Steiner et al 2010; Fischer and Glaser 2012; Liang et al 2005; Basso et al 2013). Additionally, biochar has recalcitrant properties, meaning that it is very slow to degrade and thus is a viable option for sequestering carbon in the soil as a way of off-setting atmospheric emissions (McElligott et al 2011).

However, there are very few field studies exploring the potential of biochar use in forestry in the Lake States (Minnesota, Wisconsin, and Michigan), USA. Chapter 1 aims to examine the effect that biochar and/or compost has on the growth and survival of jack pine (*Pinus banksiana* Lamb.) seedlings in northern Minnesota through a series of field experiments. This was done by conducting three field based experiments: 1) quantifying the influence of soil amendment and weed control treatments on survival and growth of jack pine seedlings; 2) quantifying the influence of soil amendments and irrigation when competition was controlled on survival and growth of jack pine seedlings; 3) quantifying the differences in planting stock – bareroot and containerized jack pine seedlings – and soil amendment in relation to jack pine survival and growth. Chapter 2 explores a topdress application treatment of biochar to a recently-harvested pine site. Biochar was spread at two different rates, with two different levels of torrefaction. This was done to learn more about what effects topdressing could have on native plant communities over time. These studies have the potential to help landowners make informed decisions about how to promote drought resilience in their forests, as well as provide a basis for long-term research projects looking into biochar use in the region’s forestry.

Chapter 1: Title: Influence of biochar, weed control, and irrigation on survival and growth of jack pine in northern Minnesota

Preface

There has been increased interest in maintaining and increasing jack pine (*Pinus banksiana* Lamb.) cover in northern Minnesota. However, successful regeneration can be difficult due to spring and summer drought conditions. One potential treatment to increase regeneration success is the addition of biochar. Biochar is a bio-based soil amendment created through pyrolysis, and has been observed to increase the water-holding capacity and cation exchange capacity of soils. Three studies were installed in northern Minnesota to explore the effects of soil amendments (including biochar) on the growth and survival of jack pine. The studies utilized a randomized factorial design with two factors in each: the first, soil amendment and weed control treatments, the second, a soil amendment and irrigation treatments, the third, containerized seedlings with a soil amendment. Results from the first study indicate that a biochar and compost amendment had the highest survival (57%) compared to compost only (48%) and no soil amendment (28%) over the first two growing seasons. Soil amendment had no effect on diameter growth, but annual weed control treatments had greater growth relative to initial weed control only. In the second study, overall survival was higher (73%) and did not significantly vary by soil amendment or irrigation. Additionally, after one growing season, neither factor significantly increased diameter or height growth. The third study found that containerized seedlings had very high survival rates regardless of soil

amendment treatments. The results from these studies will aid natural resource managers in successfully regenerating jack pine via planting.

Introduction

Increases in the global mean temperature in recent years are widely accepted to be due to anthropogenic causes (Allen et al. 2010). This shift towards a warmer climate is impacting forested ecosystems, partly because of changes in the distribution of annual precipitation (Janowiak et al. 2014; Millar et al. 2007; Weed et al. 2013). There is growing concern that an increase in extreme precipitation events brought on by climate change will result in more frequent and severe droughts, resulting in higher stress and longer recovery times in forests (Vose et al. 2016). Climate-induced drought across North America has contributed to widespread tree mortality (Allen et al. 2010; Janowiak et al. 2014). Seedlings and saplings in particular tend to be the most susceptible to drought due to poorly developed root systems creating regeneration challenges for natural resource managers (Tardif and Conciatori 2001). This has prompted land managers and researchers to explore forest management techniques focusing on resistance, resilience, and transition strategies for a changing climate (Nagel et al. 2017; Millar et al. 2007).

One tool which is hypothesized to increase resistance and resilience to drought conditions is biochar (Basso et al. 2013; Karhu et al. 2011; Spokas et al. 2009). Biochar, a soil amendment, was a staple of resource management in ancient Amazonian cultures, being termed “terra preta” or “black earth”; it was created by burying organic material and burning it, increasing soil fertility, soil organic matter content, and soil moisture availability (Lehmann et al. 2003). In the present day, biochar is still very much a part of

land management worldwide. Pedro Sanchez (2019) published a textbook about the management of soils in the tropics and discussed the use of biochar in South and Central America. Research shows that the American Indians in North America systematically burned prairies prior to European settlement in order to boost and maintain productivity (Leopold and Boyd 1999). Currently, biochar has been observed to increase crop yield in agricultural systems in the United States (Crane-Droesch et al. 2013). Present-day biochar is created when biomass undergoes a process called pyrolysis that decomposes material at high temperatures with low oxygen (Sohi et al. 2010; Lehmann and Joseph 2015).

One of the known benefits of biochar as a soil amendment is the improvement of cation exchange capacity (CEC) in soils, increasing the ability of the soil to retain nutrients, especially in sandy soils (Liang et al. 2005). Biochar has high organic matter content and good structure, allowing it to act like a “sponge” and increase water availability to seedlings (Basso et al. 2013). Thomas and Gale (2015) found that the properties and efficacy of biochar as a soil amendment varied with differing pyrolysis conditions. When wood undergoes pyrolysis at higher temperatures, it results in a biochar with higher pH and CEC, whereas biochar produced at lower temperatures tends to be more acidic (Novak et al. 2009; McElligott et al. 2011). The increased surface area due to the pyrolysis also results in increased micropores and hydrophobicity (Ahmad et al. 2014; Verhoeff et al. 2011). Biochar has a large number of open exchange sites, meaning mobile cations can be immobilized after application and potentially decrease nutrient availability in the short term (Boerner 1982). Mixing compost with the biochar is one option to ensure that the raw biochar does not immobilize all of the nutrients in the soil

available for plant growth, and therefore allow nutrients to be accessible by the seedling (Borchard et al. 2014; Steiner et al. 2010; Fischer and Glaser 2012).

While the majority of biochar research has been conducted in agricultural settings (Li et al. 2013; Omondi et al. 2016), a few studies have explored the use of biochar in forest ecosystems. McElligott et al. (2011) examined biochar as a forest soil amendment in conjunction with biomass removal in the western United States, and found potential for biochar to sequester carbon in the soil due to recalcitrant and aromatic properties that make it resistant to degradation. A meta-analysis by Thomas and Gale (2011) concluded that biochar increased biomass production by 41% on average in both tropical and boreal forests. Krapfl et al. (2016) studied the interactions between loblolly pine (*Pinus taeda* Lamb.) seedlings and switchgrass (*Panicum virgatum* Lamb.) competition with a biochar amendment which resulted in a significant increase of volumetric water content in biochar treatments. Additionally, studies done by Karhu et al. (2011) and Johnson et al. (2017) found that with the addition of biochar in agricultural soils, both CH₄ uptake and water-holding capacity of the soil increased. Recently, Richard et al. (2017) conducted a field study in the Lake States in a red pine (*Pinus resinosa* Aiton.) system with well-drained sandy loam/loamy sand, and found that soil structure, soil acidity, cation exchange capacity, and water-holding capacity were improved with biochar addition. In a meta-analysis, coarse soils such as sands have been found to have increased productivity with the addition of biochar due to the increase in water-holding capacity (Jeffery et al. 2011). Given the above, biochar additions may increase water availability during periods of drought, and increase the survival and growth of seedlings, especially in sandy, coarse soils.

In addition to drought stress, aboveground competition is a limitation to seedling establishment. Competition control such as the removal of non-desired vegetation (weeds) through mechanical (e.g. brush-cutter) or chemical (e.g. herbicide) methods is a common silvicultural practice to increase growing space for seedlings (Nyland 2002). Herbaceous vegetation competition decreases availability of soil water during dry months in multiple systems (Harrington 1991; Davis et al. 1998). These studies both used a glyphosphate herbicide, but research has shown that manual weed removal can also be effective at removing competing woody and non-woody vegetation in order to release seedlings (Bell et al. 1997).

Outwash, coarse sandy soils are a prominent soil type of the US Great Lakes Region (Minnesota, Michigan, Wisconsin) due to the past glacial history of the region (Anderson et al. 1999). These sandy soils are excessively drained and nutrient poor. Jack pine (*Pinus banksiana* Lamb.) is one of the few tree species that can survive under these harsh conditions. This species typically has serotinous cones that require fire and heat to release the seeds, and fire has historically been a natural part of their ecosystem in the northern Lake States (Godbout et al. 2005). Jack pine is shade intolerant and is often a pioneer species on recently disturbed sites where seedlings germinate best on exposed mineral soil in full sunlight without competing vegetation (Benzie 1977; Carey 1993). Higher amounts of early spring precipitation and drier late summers have been found to favor seedling establishment (Tardif and Conciatori 2001). Jack pine forests currently cover about 141,640 hectares (roughly 2%) of forested land in Minnesota, and are economically important for providing valuable forest products as well as filling an essential ecological niche (Miles 2017). The current extent has decreased from pre-

European settlement forests in northern Minnesota, where more frequent fires occurred than occur today, creating an environment suitable for establishment of jack pine forests and barrens. Fires would have naturally added a charcoal layer to the soil, decreased competition, and aided mineral soil exposure and heat for natural regeneration of jack pine (Sohi et al. 2010).

The long-term goals and objectives of this project are to assess if a biochar and compost addition to a sandy soil influence survival and growth of jack pine seedlings. The aim is to increase the survival of seedlings to help the forest maintain resilience under summer drought conditions that are predicted to be more frequent with a changing climate. In this context, resilience is defined as how well the seedling is able to withstand disturbance (DeRose and Long 2014). This project will examine how short-term survival and growth of jack pine seedlings are affected by soil amendments such as biochar and compost, and determine how plant competition and irrigation influences the response. By understanding the multiple factors which influence seedling growth and survival, natural resource managers can prioritize treatments which increase resistance and resilience to changing environmental conditions.

Methods

Three distinct but parallel studies were initiated in northern Minnesota that explored the effects of biochar and compost amendments on the growth and survival of jack pine seedlings; one study looked at bare root seedlings and weed control treatments, one looked at bare root seedlings and irrigation treatments, and one looked at containerized seedlings.

Weed Control Study – Area

The first study took place in the Superior National Forest (henceforth SNF) in northeastern Minnesota, USA. The SNF is approximately 1.6 million hectares in size and lies on the transition zone of broadleaf deciduous and northern boreal forests. Common species of the broadleaf forest in this area are paper birch (*Betula papyrifera* Marsh.), maple (*Acer* spp.), and basswood (*Tilia americana* Lamb.); common species in the boreal forests are jack pine, red pine, and black spruce (*Picea mariana* Mill.). This transition zone creates unique forest communities composed of species dominant in both zones (US Forest Service). Historically, management in the SNF favored red pine and quaking aspen (*Populus tremuloides* Michx.) as merchantable species in place of jack pine, but management is currently more focused on restoring jack pine. However, in recent years, jack pine has shown an increase in drought-related mortality (Peng et al. 2011). In a 2017 survey given to forest agencies in Minnesota, jack pine was ranked as the most difficult species to regenerate (Windmuller-Campione et al. 2019).

The study sites occurred on sandy, nutrient poor outwash plains and glacial moraines commonly dominated by pine species. This region is characterized by depositional sediments sourced from the last glaciation and associated landforms (Ojakangas and Matsch 1982). The mean annual temperature is 2-3 °C with 70 cm of precipitation, with 165 cm falling as snow (snow water equivalent = 17 cm; Figure 1.1; NOAAa; NOAAb). The growing season is short, typically running from May to mid-October.

Weed Control Study – Experimental design:

Three sites were selected from a candidate list of sites that were scheduled to be planted with jack pine by the Superior National Forest (Table 1.1).

Table 1.1. Summary information about the three selected sites for the Weed Control Study

Site	Coordinates (Latitude, - Longitude)	Pre- harvest cover type	Silvicultural System	Post-harvest treatment	Hectares harvested	Soil type
Standard site prep site (SS)	47.602057, - 92.808743	Quaking aspen	Seed tree	Slash was left in piles	4.50	Greatscott-Nashwauk- Balkan complex: stony loam, depressional
Fresh slash site (FSS)	47.603004, - 92.805684	Quaking aspen, white spruce	Clearcut	Slash was scattered and planted immediately	4.82	Beargrease series: well- drained, very stony loam
Burned Site (BS)	47.669101, - 92.811013	Jack pine	Clearcut	Site was burned following harvest	8.10	Graycalm-Biwabek complex: excessively drained, loamy sand

Soil data from Web Soil Survey

At each site, seedlings were planted in a randomized factorial design with two factors. The experimental unit was individual jack pine seedlings (2-0 bare root) sourced from the MN DNR nursery and planted in May of 2016 in a 2x3 meter grid spacing. The first factor was soil amendment with three levels: Biochar+Compost (1 liter mix with equal parts biochar and compost), Compost-only (0.5 liter), and a No Amendment added. Amendments were applied by placing the material at the base of the planting hole. Biochar was created from Rocky Mountain lodgepole pine (*Pinus contorta* Doug.). The second factor was weed control with two levels (either initial (IWC) or annual (AWC) application). All seedlings received IWC in July 2016 (manual control with Stihl FS 250 brush saws), and those assigned to the AWC treatment received treatment multiple times throughout the subsequent growing seasons using the same equipment (2016 – 2018) (Table 1.2). Manual weed control methods were used since no chemical treatments are permitted on the national forests in the Great Lakes Region. Each treatment combination was replicated 15 times (n = 90 at each site). All seedlings were protected from deer browse using mesh tubing (Rigid Seedling Protector Tubes from Forestry Suppliers) held in place with bamboo stakes. Seedlings that suffered mortality in the first growing season (2016) were replanted during the spring of 2017 using the same stock type and nursery (MN DNR nursery).

Table 1.2. Timeline of events for Weed Control Study

Action	Time accomplished
Planting	May 2016 and 2017
Weed control	Early summer and mid-late summer 2016, 2017, 2018
Growth/survival measurement	May 2016, 2017, and October 2016, 2017, 2018

At the time of planting, the height (measured in centimeter to both the bud tip and extended needle) and basal diameter (two dimensions measured with calipers to nearest 1/10 mm) of each seedling was recorded. After the seedlings hardened at the end of each subsequent growing season (mid-October), mortality status was recorded and heights and basal diameters were measured. Annual growth was determined by subtracting the previous measurement from the most recent measurement.

Irrigation Study – Area:

Since competition was observed to be a factor influencing growth, another experiment was conducted where competition was completely controlled in order to evaluate the effects of soil amendment and irrigation on survival and growth of jack pine. This experiment was installed at the University of Minnesota's Cloquet Forestry Center (hereafter CFC), which is located 43 kilometers southwest of Duluth, Minnesota, USA in Carlton County. The mission of the CFC is to provide teaching, research, and outreach to the state of Minnesota and the region regarding forest management, forest ecology, and other related fields. There are full time staff and researchers at the CFC, which allowed for application of the irrigation treatments.

Irrigation Study - Experimental design:

The experiment was conducted on Omega loamy sand soils that were previously occupied by a mix of conifer species including jack pine, red pine, and balsam fir (*Abies balsamea* Lamb.). All overstory and understory trees were removed in the spring of 2017; the site was prepped with a roller-disk, providing a completely clean planting area. Eighty bare root jack pine (2-0 stock) supplied from the MN DNR nursery were planted on a 3x3 meter grid in a randomized factorial design with two factors (1:soil

amendment and 2: irrigation). The soil amendment factor had four levels: Biochar-only (0.5 liter), Compost-only (0.5 liter), Biochar+Compost (1 liter mix with equal parts biochar and compost), and a No Amendment. The irrigation factor had two levels: ambient water via precipitation or irrigated (Water and No Water Added) (Figure 1.1). In the Water treatment, seedlings received an additional three liters of water by hand three times per week during the growing season (Table 1.3). Throughout the growing season, a cornerstone glyphosate herbicide was applied to continually eliminate any competing vegetation within the study area. All seedlings were protected from deer browse using mesh tubing (Rigid Seedling Protector Tubes from Forestry Suppliers) held in place with bamboo stakes.

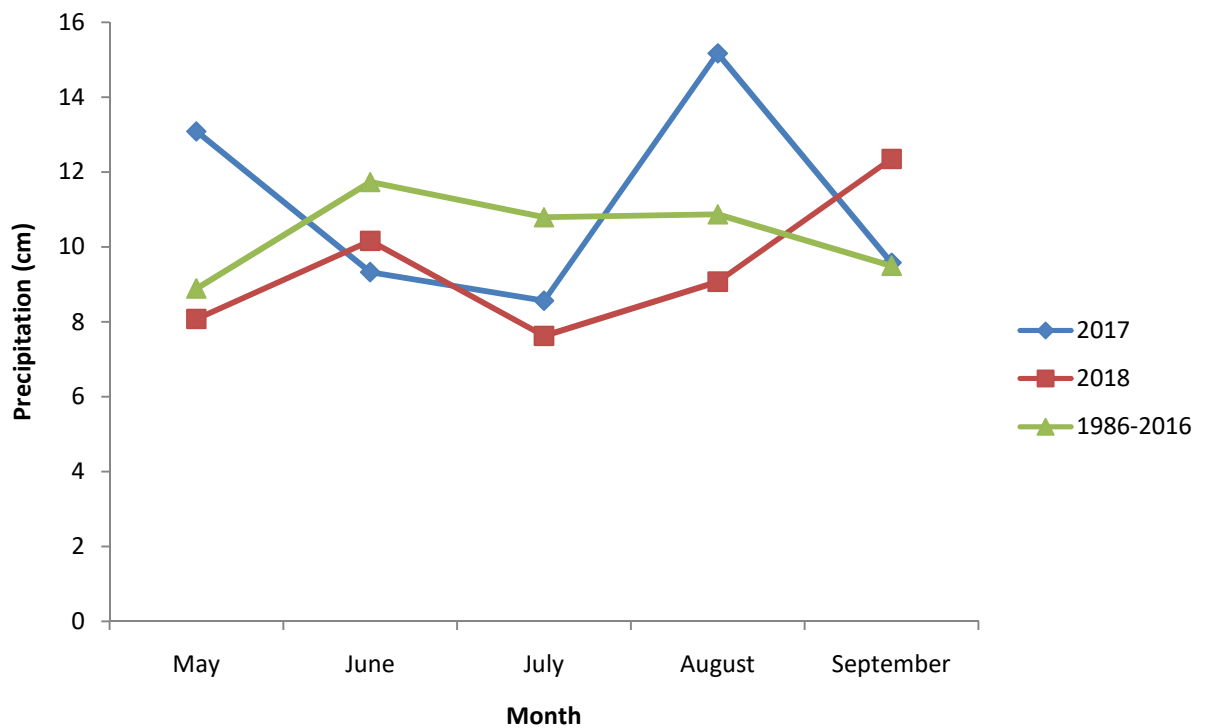


Figure 1.1. Precipitation data from Cloquet Forestry Center, adjacent to Irrigation study plots (data accessed through xmACIS2, NOAA Regional Climate Centers), showing precipitation (cm) during growing season (May-September) from 1986-present.

Table 1.3. Timeline for Irrigation Study

Action	Time accomplished
Planting	June 2017, May 2018
Growth and survival measurements	June 2017, May 2018, October 2017-18
Irrigation treatment	M-W-F May-August, T-Th September 2017-18
Herbicide application	Throughout growing season

At the time of planting, basal diameters (mm) and the height (cm) to the extended bud and needle of the seedlings were collected (using calipers and a meter stick, respectively).

This was also done at the end of the growing season (mid-October).

Containerized Study – Area:

A third paired study was established at the CFC to explore the influence of stock type with soil amendments. Bare root seedlings are commonly used in planting in the region due to both expense and logistics. However, containerized stock have increased in availability and in other regions (Intermountain West) have shown increased survival under summer drought conditions compared to bare root (Brissette et al. 1991).

Containerized Study – Experimental Design:

Forty containerized jack pine (2-0) seedlings (North Central Reforestation, Inc.) were planted in the spring of 2017 on a 3x3 meter grid spacing in a randomized design with four soil amendment treatments: Biochar-only, Biochar+Compost, Compost-only, and a No Amendment. Application rate was 0.5 liters of biochar and compost each, and a 1:1 ratio mix of Biochar+Compost (1 liter total) poured directly into the hole that the seedling plug was planted in. A cornerstone glyphosphate fertilizer was applied throughout the growing seasons to ensure elimination of competition.

Data Analysis:

Diameter values were taken as the average of the two dimensions measured, while height values were the average of the bud height and needle height at the time of planting and at the end of each subsequent growing season. Relative annual growth rates were calculated for each seedling (example: $[(2018 \text{ measurement} - 2017 \text{ measurement}) / 2017 \text{ measurement}] \times 1 \text{ year}$) and run with the 'ggplot2' R package (Wickham et al. 2018).

Data was normally distributed so no transformations were performed. Data on seedling growth was analyzed with Generalized Linear Mixed (GLM) Models using R statistical software (R Core Team 2017). Effects used were those of treatment (Biochar+Compost, Compost-only, and No Amendment), weed control (AWC and IWC), and site (SS, FSS, and BS) as fixed effects when applicable. Significance levels were set at 0.1 due to high variability in the response variables. When interactions among the fixed effects were observed (weed control, soil amendment, and site), differences among treatment combinations were tested using least squared differences (LSD) within the 'agricolae' and 'car' R packages (Fox et al. 2018; Mendiburu 2019).

Logistic regression was used to assess seedling survival for the Weed Control Study. The response variable of seedlings surviving from the start of the study to the end of the third growing season was binary (0=dead, 1=alive). Logistic regression of survival was plotted against initial height of the seedlings, since that was found to be a significant variable influencing growth. Because of low survival, ANOVA was not used to assess treatment effects.

Similar methods were applied for the Irrigation and Containerized Studies at the CFC including the relative annual growth rates, the use of GLM models, and logistic

regression for survival. For individual trees that decreased in relative diameter (<0.1 mm) from the start to the end of the growing season, values (14 total) were adjusted to reflect no change or the diameter value was estimated as the average between prior and subsequent measurements due to a margin of measurement error. Seedlings were excluded from analysis if there was more than a -0.1 mm relative difference within the growing season (11 seedlings).

Results

Weed Control Study – 3 Year Survival Data:

After three years, seedling survival was low across all sites and treatments; total survival across all sites regardless of treatment was only 41% ($n=270$) and ranged from a low of 34% at the fresh slash site (FSS) to a high of 49% at the burned site (BS) (Table 1.4). FSS had both the lowest and highest survival of seedlings for individual treatment combinations; the FSS site had only 7% of seedlings survive in the No Amendment with annual weed control (AWC) treatment and 67% in the Biochar+Compost+AWC treatment. Across all sites and after three years, the Biochar+Compost and Compost-only treatments had more than double the number of seedlings survive (46 and 42 respectively, out of 90) compared to the No Amendment (24 out of 90). This trend was similar within sites, where the Biochar+Compost and Compost-only amendments had consistently higher survival than the No Amendment (Table 1.4). Initial seedling height was the only significant predictor of survival in a logistic regression ($p = 0.05$); the taller the seedling at initial time of planting, the greater probability of surviving to year 3 (Figure 1.2).

Table 1.4. Weed Control Study seedling survival (in percentage) after 3 years. Site totals and amendment totals are underlined.

	Biochar+Compost	Compost-only	No Amendment	Total
Standard Site	<u>50%</u>	<u>40%</u>	<u>33%</u>	<u>41%</u>
Weed Control	40%	33%	27%	33%
No Weed Control	60%	47%	40%	49%
Fresh Slash Site	<u>57%</u>	<u>33%</u>	<u>13%</u>	<u>34%</u>
Weed Control	67%	40%	7%	38%
No Weed Control	47%	27%	20%	31%
Burned Site	<u>47%</u>	<u>67%</u>	<u>33%</u>	<u>49%</u>
Weed Control	47%	60%	40%	49%
No Weed Control	47%	73%	27%	49%
Total	<u>51%</u>	<u>47%</u>	<u>27%</u>	<u>41%</u>

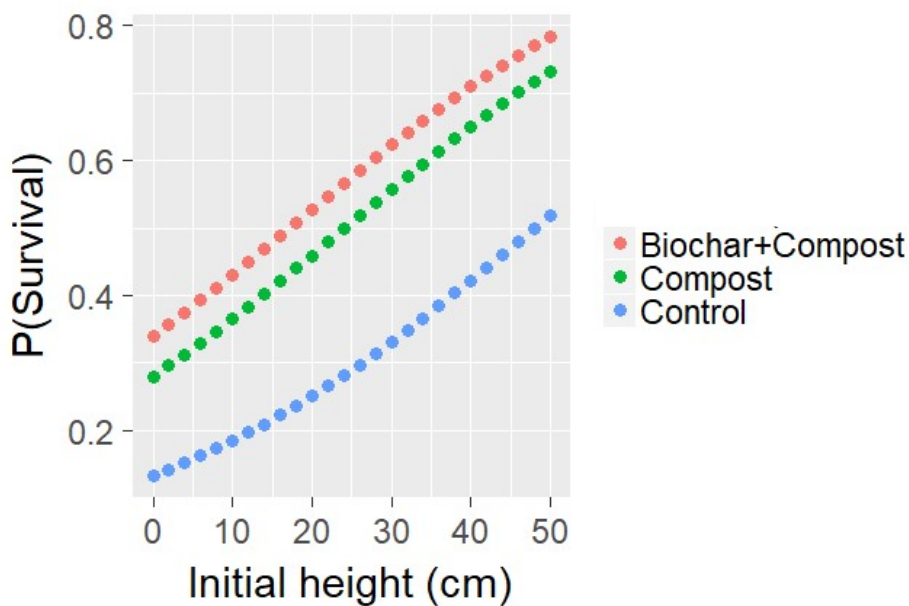


Figure 1.2. Weed Control Study probability of survival across all sites by soil amendment treatment as a function of initial height. There were no significant differences in survival among treatments and sites within Weed Control Study.

Weed Control Study – Diameter and Height Growth:

Treatment effects on mean relative annual diameter growth and annual height growth were variable across years and treatments (Table 1.5). There were significant interactions between soil amendment and weed control for relative diameter growth in 2017 ($p=0.07$). The combination of Biochar+Compost with AWC resulted in significantly greater diameter growth (2017) in jack pine compared to jack pine treated with No Amendment of either AWC ($p=0.01$) or IWC ($p=0.03$; Table A1.1). Significant differences were also observed among the Compost-only AWC treatment and No Amendment with IWC ($p<0.01$), Compost-only with IWC ($p=0.05$), and No Amendment with IWC ($p=0.01$; Table A1.1). Seedlings planted with Compost-only experienced greater growth than those planted with No Amendment, regardless of AWC or IWC.

As for main effects, there was also a significant effect of site on diameter in 2016 ($p=0.01$; Table 1.5) which manifested as relative diameter growth at BS being significantly greater than SS (Table 1.6). Effect of weed control on diameter was significant in 2016 and 2018 ($p=0.02$ and $p<0.01$, respectively; Table 1.5). There were consistent significant differences between the weed control treatments for diameter growth for all three years; seedlings in the AWC treatments had greater diameter growth compared to the IWC treatment (2016 – $p=0.02$, 2017 – $p=0.02$, 2018 – $p<0.01$), although the effects in 2017 are likely due to the aforementioned interaction between weed control and soil amendment in that year (Figure 1.3).

An interaction was found between soil amendment and weed control for relative height growth in 2018 ($p=0.10$; Table 1.5), however, multiple comparisons failed to detect significant interactions. This is in addition to soil amendment and weed control effects in

2018 for height, and significant differences between sites for height growth in 2018 (Table 1.5). This manifested as SS having significantly higher growth than FSS and BS (Table 1.7).

Table 1.5. Results of individual ANOVA tests for factors and interactions of Weed Control Study.
 Bolded values are significant at p=0.1. WC=Weed Control and Amend=Amendment treatment.

	Amend		WC		Site		WC*Amend		WC*Site		Amend*Site		WC*Amend*Site	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
2016														
diameter	0.22	0.80	5.44	0.02	4.67	0.01	0.18	0.84	1.09	0.34	1.68	0.16	0.59	0.67
height	0.64	0.53	1.57	0.21	2.02	0.14	1.14	0.32	0.60	0.55	0.73	0.57	0.65	0.62
2017														
diameter	7.81	<0.01	5.24	0.02	0.40	0.67	2.68	0.07	0.93	0.40	1.79	0.13	0.62	0.65
height	8.17	<0.01	4.84	0.03	0.55	0.58	0.97	0.38	1.76	0.17	0.81	0.52	1.13	0.34
2018														
diameter	0.45	0.64	9.14	<0.01	0.58	0.56	1.09	0.34	1.19	0.31	0.48	0.75	1.46	0.21
height	0.29	0.75	0.77	0.38	6.28	<0.01	2.35	0.10	0.65	0.52	1.10	0.36	1.61	0.17

Table 1.6. Mean relative annual diameter growth (mm/mm) of main effects (standard errors in parentheses). Significance between weed control treatments denoted as *=significant. Differences among sites marked with capital letters when significant. Only the main effects are displayed. Table 1A displays interaction effects in 2017.

Site	IWC				AWC		Biochar+Compost		Compost-only		No Amendment		
2016													
	SS	B	0.17	(0.07)		0.42	(0.12)	0.21	(0.05)	0.42	(0.18)	0.19	(0.07)
	FSS	AB	0.41	(0.09)		0.45	(0.06)	0.38	(0.05)	0.51	(0.14)	0.48	(0.17)
	BS	A	0.46	(0.05)		0.57	(0.08)	0.61	(0.11)	0.42	(0.05)	0.54	(0.99)
	All sites		0.34	(0.04)	*	0.50	(0.05)	0.41	(0.05)	0.44	(0.06)	0.38	(0.06)
2017													
	SS		0.12	(0.04)		0.22	(0.06)	0.19	(0.06)	0.25	(0.06)	0.06	(0.06)
	FSS		0.11	(0.05)		0.28	(0.07)	0.36	(0.07)	0.19	(0.07)	0.01	(0.07)
	BS		0.14	(0.05)		0.16	(0.05)	0.14	(0.07)	0.21	(0.06)	0.09	(0.06)
	All sites		0.12	(0.03)	*	0.22	(0.03)	0.23	(0.39)	0.22	(0.04)	0.06	(0.04)
2018													
	SS		0.28	(0.03)		0.45	(0.06)	0.35	(0.04)	0.37	(0.07)	0.37	(0.08)
	FSS		0.22	(0.06)		0.42	(0.06)	0.35	(0.07)	0.32	(0.07)	0.28	(0.09)
	BS		0.35	(0.05)		0.38	(0.05)	0.30	(0.06)	0.45	(0.07)	0.33	(0.06)
	All sites		0.28	(0.03)	*	0.42	(0.03)	0.39	(0.04)	0.38	(0.04)	0.33	(0.04)

Table 1.7. Mean relative annual height growth (cm/cm) of main effects (standard errors in parentheses). Significance between weed control treatments denoted as *=significant. Significance between soil amendments denoted with lower case letters of significance, and differences between sites marked with capital letters. Only the main effects are displayed. Multiple comparisons failed to detect significant differences among treatment combinations in 2018.

Significant differences among treatment combinations in 2016												
		IWC		AWC		Biochar+Compost		Compost-only		No Amendment		
2016												
	SS	0.26	(0.05)	0.39	(0.08)	0.38	(0.09)	0.34	(0.08)	0.18	(0.07)	
	FSS	0.22	(0.05)	0.26	(0.06)	0.23	(0.05)	0.26	(0.08)	0.19	(0.11)	
	BB	0.18	(0.07)	0.23	(0.03)	0.19	(0.06)	0.21	(0.07)	0.21	(0.09)	
	All sites	0.22	(0.04)	0.28	(0.03)	0.26	(0.04)	0.26	(0.04)	0.20	(0.05)	
2017												
	SS	0.51	(0.08)	0.28	(0.08)	0.53	(0.09)	0.39	(0.09)	0.28	(0.11)	
	FSS	0.43	(0.09)	0.46	(0.10)	0.69	(0.11)	0.49	(0.13)	0.14	(0.07)	
	BS	0.62	(0.12)	0.36	(0.08)	0.57	(0.11)	0.58	(0.13)	0.31	(0.12)	
	All sites	0.52	(0.05)	0.36	(0.05)	0.60	(0.58)	a	0.48	(0.07)	a	
2018												
	SS	A	0.66	(0.07)	0.70	(0.08)	0.78	(0.09)	0.61	(0.11)	0.67	(0.07)
	FSS	B	0.43	(0.08)	0.56	(0.08)	0.47	(0.09)	0.58	(0.12)	0.44	(0.09)
	BS	B	0.43	(0.06)	0.38	(0.07)	0.31	(0.07)	0.49	(0.07)	0.41	(0.10)
	All sites		0.51	(0.04)	0.57	(0.05)	0.54	(0.53)	0.56	(0.06)	0.50	(0.05)



Figure 1.3. Annual relative diameter growth by weed control treatment across all sites for 2016, 2017, and 2018. Differences between treatments were significant in each year. There was an interaction between weed control and soil amendment affecting diameter growth in 2017.

Irrigation Study – Bare root seedlings – Survival & Growth:

After two years, survival was relatively high (70%) across all treatments (Table 1.8). The No Amendment treatment for both irrigation treatments had 90% survival compared to only 50% and 60% survival in the Biochar+Compost treatment with either no irrigation or irrigation added, respectively (Table 1.8). Results from a logistic regression indicated that there were no significant effects of soil amendment or irrigation treatment on survival, but seedlings planted with a larger diameter had a higher probability of survival ($p=0.07$) (Figure 1.4). Mean relative annual diameter and relative annual height growth varied among years and treatments (Table 1.10). Soil amendment had a significant effect on relative diameter growth in 2017 ($p=0.06$), with Biochar-only having significantly

higher growth than the Compost-only amendment. There was an interaction between soil amendment and irrigation with seedling diameter in 2017 ($p=0.05$) (Table 1.9), however, multiple comparisons failed to detect significant differences among treatments. Negative values were observed for relative annual height growth during 2017 due to die-back and subsequent reflushing (Table 1.10).

Table 1.8. Two-year Jack pine survival by main treatment factor for the Irrigation study.

	Biochar-only	Compost-only	Biochar+Compost	No Amendment	Total
No water	60%	70%	50%	90%	68%
Water	70%	70%	60%	90%	73%
Average	65%	70%	55%	90%	70%

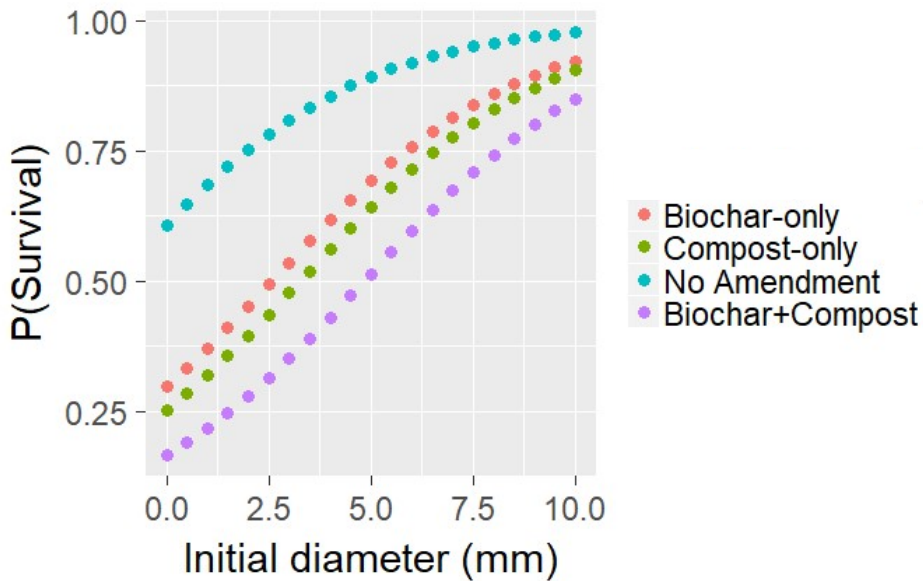


Figure 1.4. Probability of survival after two years at Irrigation study by soil amendment as a function of initial diameter.

Table 1.9. Results of individual ANOVA tests for factors and interactions of Irrigation Study.
 Bolded values are significant at p=0.1

	Amendment		Irrigation		Amend*Irrig	
	F	P	F	P	F	p
2017						
diameter	2.68	0.06	0.01	0.92	0.82	0.05
height	1.78	0.17	0.74	0.39	1.43	0.25
2018						
diameter	0.68	0.57	2.36	0.13	0.10	0.96
height	0.73	0.54	0.12	0.74	0.10	0.96

Table 1.10. Mean relative annual diameter growth (mm/mm) and height growth (cm/cm) of main effects (standard errors in parentheses) for each year of Irrigation Study. Multiple comparisons failed to detect significant differences among treatment combinations.

	No water		Water		Biochar-only	Compost-only	Biochar+Compost	No Amendment
2017								
diameter	0.20	(0.05)	0.20	(0.06)	0.33 (0.12)	0.07 (0.03)	0.13 (0.05)	0.25 (0.05)
height	0.19	(0.20)	0.0	(0.03)	0.45 (0.36)	-0.05 (0.06)	-0.07 (0.09)	0.06 (0.04)
2018								
diameter	0.5	(0.06)	0.65	(0.07)	0.63 (0.9)	0.57 (0.06)	0.45 (0.09)	0.66 (0.14)
height	1.23	(0.15)	1.31	(0.16)	1.34 (0.21)	1.45 (0.26)	0.98 (0.19)	1.27 (0.20)

Containerized Study – Survival& Growth:

Survival of containerized seedlings was high after two years (86%). The control treatment had 100% of seedlings survive; 70% of seedlings survived in the Biochar+Compost treatment, and the Biochar-only and Compost-only treatments both had 90% survival. There were no significant differences among surviving individuals for annual relative diameter or height growth (Table 1.12). Negative height growth was observed in 2017 which was due to dieback and subsequent reflushing. In 2018, seedlings grew an average relative amount of 0.92 mm in diameter and 0.55 cm in height across treatments (Table 1.11 & Table 1.12).

Table 1.11. ANOVA-derived F-statistics and associated p value significance for Containerized Study. No p values are of significance at alpha=0.1

	Amendment	
	F	P
2017		
diameter	1.05	0.38
height	0.66	0.58
2018		
diameter	0.74	0.53
height	0.84	0.48

Table 1.12. Mean relative annual diameter growth (mm/mm) and height growth (cm/cm) for containerized seedlings by treatment and year of the Containerized Study. No significant treatment effects were found so only the main effect means are displayed.

	Biochar-only		Compost-only		Biochar+Compost		Control	
2017								
diameter	0.23	(0.14)	0.45	(0.24)	0.64	(0.32)	0.82	(0.70)
height	-0.01	(0.01)	-0.04	(0.01)	-0.05	(0.02)	-0.06	(0.05)
2018								
diameter	1.21	(0.14)	0.78	(0.23)	0.72	(0.28)	0.96	(0.28)
height	0.89	(0.22)	0.45	(0.17)	0.72	(0.23)	0.67	(0.18)

Discussion

Biochar as a soil amendment influences the properties of soils; it has been observed in certain systems to improve CEC, water holding capacity, nutrient availability of a soil, and increase long-term carbon storage (Liang et al. 2005; Basso et al. 2003; McElligott et al. 2011). However, the effects that a biochar amendment addition has on forest soils and seedling resistance and resilience to drought still need to be explored. Results from this study indicate that short-term effects of biochar and compost amendments on jack pine seedling survival and growth in northern Minnesota were variable.

Biochar is widely used as a way to boost agricultural crop productivity (Barrow 2012). However, agricultural systems are often managed on an annual basis, meaning their soil fertility requirements are very different than that of forests, which often take decades of growth before harvest. Research on biochar in forest systems has shown to be more variable, possibly in part due to the long range of time needed to grow enough biomass to determine quantifiable effects (Thomas and Gale 2015).

Similar to what Richard et al. (2017) observed, biochar and compost amendments did not have a significant effect on the growth of seedlings. This is contrary to another study in the western United States that observed a positive effect of biochar on seedling growth, in part due to increased CEC and lower C:N ratios (Robertson et al. 2012). Still other studies have found a negative effect on seedling growth (Glaser 2002; Deenik 2010), believed to be a result of a spike in microbial activity in biochar-amended soils, leading to net N immobilization (Kolb et al. 2009; Deenik 2010; Verheijen et al. 2010). While soils were not chemically tested here, conclusions can be drawn from the data that there

was no significant difference in growth and survival between the biochar and compost amendments. Rather than amendment addition, competition removal had the largest effect on early seedling survival and growth.

It is important to consider the influence of vegetation competition and its effects on seedling growth and survival. Interspecific competition is known to have negative effects on the growth of forest conifers (Walstad and Kuch 1987). Here, weed control was observed to have a greater influence on seedling growth and survival than soil amendments in the Weed Control Study on the SNF. This is in agreement with what Miller et al. (1991) observed in the importance of herbaceous and woody competition control for early seedling survival and growth in southeastern forests, and Wagner (2000) observed similar effects in northern boreal forests. It should be noted that both the southeastern US and the Pacific Northwest are generally highly productive systems, with certain pine systems having high management intensity and short rotation ages (Fox et al. 2007; Johnson et al. 2005). When competition was removed in the Irrigation Study, seedlings had higher survival regardless of amendment treatment, providing additional indication on the importance of competing vegetation control during seedling establishment. Given this, both aboveground and belowground competition may be a limitation to establishing jack pine in previously fire-dominated systems with current fire suppression. Historically, jack pine seedlings established after mixed severity or stand replacing fires which would reduce competing vegetation, expose mineral soils, and add nutrients through burned material into the soil (Johnson et al. 2007). Jack pine seedlings have been shown to regenerate best when they don't have to compete for light; Longpre et al. (1994) identified that jack pine did not grow as well when it had to compete with

aspen for sunlight, as aspen easily dominates jack pine (Farmer et al. 1988). In the Weed Control Study, the FSS had a large amount of aspen regenerating among the planted jack pine.

What was found to be significant, however, was that seedlings that were planted with a Biochar-only or a Biochar+Compost amendment and received weed control had higher rates of diameter growth during their second year than those planted without amendments, regardless of whether treated with weed control or not. However, this relationship was not observed at the Irrigation study. Schmidt et al. (2014) compared Biochar-only amendments and Biochar+Compost amendments, and found that Biochar+Compost outperformed the Biochar-only amendment only in the first year. Thus there is high variability in studies on short-term results of soil amendments on seedling growth.

Studies have found that woody crops (such as grapes) growing in temperate climates did not respond as well to biochar soil additions compared to similar studies conducted in tropical regions (Schmidt et al. 2014; Teat et al. 2015). It is hypothesized that this is because the biochar can be resistant to nutrients, further overcome by adding compost to alter the negative hydrophobic characteristics of biochar and increase surfaces that absorb nutrients (Ding et al. 2010; Taghizadeh-Toosi et al. 2011; Borchard et al. 2014; Steiner et al. 2010; Fischer and Glaser 2012; Pietikainen et al. 2003; Cheng and Lehmann 2009; Zimmerman 2010). This is why the Irrigation Study was designed to look at Biochar-only, Compost-only, and Biochar+Compost amendments.

Given the importance of water availability to seedling survival (Hébert et al. 2006), the lack of irrigation effect on seedling growth and survival is puzzling. In the Irrigation Study, water was poured from a bucket around the base of the seedling. Due to rapid drainage of the soil at the site (USDA Web Soil Survey), the irrigated water may have drained before fully coming in contact with the soil amendment or roots. It also could have partially evaporated before completely permeating the soil on warmer days. These scenarios could lend to the lack of effect (or inverse effect) of irrigation on seedling growth and survival shown by the results (Table 1.10). The Irrigation Study presented here found that amendment and irrigation had a limited effect on survival and growth. This is hypothesized to be due to the fact that competition appeared to be the largest factor affecting early growth, and the growing seasons of the study both had months that were wetter than average (Figure 1.1). These limitations may be part of the reason for inverse survival. However, it will be important to look at longer term effects on how water, competition control, and soil amendment influence longer term dynamics.

The type of stock used during planting is also important to consider. Containerized seedlings are becoming more common across the United States in forest management due to increased survival, especially on adverse sites (South et al. 2005). In the Containerized Study all seedlings, regardless of soil amendment, had high survival and no replanting was required. While bare root seedling plugs are cheaper to purchase upfront, the Containerized Study shows that containerized seedlings have higher survival rates after the first few years of growth, resulting in higher yield and less costs on replanting. This is likely due to the fact that they, being already contained in soil, experience less relocation shock, therefore easing the transition into forest soils (South et al. 2005).

One consistent effect observed across all three studies was that larger seedlings have a higher probability of survival that increased with increasing initial diameter or height. Slash pine (*Pinus elliottii* Engelm.) seedlings planted at a root collar diameter of 4.5 mm or greater had a 75% chance of survival (South and Mitchell 1999). Root-mass ratio has been shown to be closely related to the survival of southern pine seedlings, as is the ability to grow new roots after transplanting (Larsen et al. 1986). This ratio was not measured in either of the three studies presented here, but could have to do with the higher survival in the Containerized Study, as the seedlings were able to grow new roots within the soil they came in, boosting their productivity before coming into contact with the sandy soil on the site or the applied amendments. All three studies had higher rates of survival with larger planted seedlings.

While there was variability among three biochar studies presented here, the use of biochar as a soil amendment did not significantly reduce short-term growth or survival. Thus, biochar may be a surrogate for fire in fire dependent or fire adapted systems like jack pine forests in the Great Lakes. Matovic (2011) states that in localized uses, the ecological impact of spreading biochar would be similar to that of a wildfire, except that more of the carbon would be retained in the soil rather than escaping into the atmosphere. This could be a viable option for sequestering carbon and off-setting anthropogenic emissions. Charcoal in fire-prone soils often makes up 5-15% of the total burned biomass, likely having significant effects on soil properties, although these effects are unclear in managed forests where fire has been suppressed (Santin et al. 2016; Deluca and Aplet 2008). Additionally, biochar may be a viable option to add to forest systems to increase long-term carbon storage without having harmful consequences on regenerating

seedlings (McElligott et al. 2011). More field-based forestry research is needed on biochar's impact on native plant communities, economic cost-benefit analysis of the treatment, and long-term effects.

Conclusions and Implications

With an increase in growing season drought severity predicted in the coming years, it is essential for land managers to practice adaptive management and experiment with ways to improve their forest's regeneration, resilience, and transition strategies (Nagel et al. 2017). Entities in Northern Minnesota, USA have reported jack pine regeneration failure due to drought stress (Windmuller-Campione et al. 2019), which served as the basis for this study to explore the potential of using biochar as a soil amendment to increase seedling survival and growth.

The Weed Control Study found that periodically removing competition manually throughout the growing season did increase seedling growth, significantly so when the seedling was planted with either a Biochar+Compost or a Compost-only amendment. Additionally, the soil amendment significantly increased survival across all sites. With this in mind, the Irrigation Study results were much more variable, showing that neither the soil amendment nor the irrigation treatment had a significant effect on growth and survival of the jack pine seedlings. Overall, they had much higher survival than those planted for the Weed Control Study. The Containerized Study had much higher survival than that of both the Weed Control Study and the Irrigation Study. There was little to no effect of amendment on the growth and survival of the containerized seedlings after two years.

Combined, these three studies look at various interacting effects of using biochar and compost amendments when planting seedlings. Overall, it appears that controlling competing vegetation is the most important factor in aiding seedling regeneration and resilience during the first few years, providing they have adequate water. If implementing a weed control regime, seedlings may benefit from a soil addition of biochar or compost.

At present, there are very few field studies using biochar as a forest soil amendment. It is the hope that the results of this study can aid other researchers and land managers looking to experiment with biochar. Increasing the use of biochar in forested landscapes can sequester carbon and offset CO₂ emissions, as well as potentially help tree seedlings to grow, when implemented with a weed control regime.

Appendix

Table A1.1. Results from Weed Control Study pairwise multiple comparisons. Relative annual diameter growth rate in diameter of seedlings in 2017. Standard errors are in parentheses. There were significant interactions between weed control and soil amendment treatments. The combination of Biochar+Compost with AWC resulted in significantly greater growth and jack pine treated with no amendment and either AWC ($p=0.01$) or IWC ($p=0.03$). Other significant differences arose among the Compost-only AWC treatment and No Amendment with IWC ($p<0.01$). Compost-only with IWC ($p=0.05$), and No Amendment with IWC ($p=0.01$).

Contrast	Estimate	p.value
Biochar+Compost,AWC - Compost-only,AWC	-0.03 (0.07)	1.00
Biochar+Compost,AWC - No Amendment,AWC	0.25 (0.07)	0.01
Biochar+Compost,AWC - Biochar+Compost,IWC	0.12 (0.07)	0.56
Biochar+Compost,AWC - Compost-only,IWC	0.17 (0.07)	0.17
Biochar+Compost,AWC - No Amendment,IWC	0.22 (0.07)	0.03
Compost-only,AWC - No Amendment,AWC	0.29 (0.07)	<0.00
Compost-only,AWC - Biochar+Compost,IWC	0.16 (0.07)	0.27
Compost-only,AWC - Compost-only,IWC	0.21 (0.07)	0.05
Compost-only,AWC - No Amendment,IWC	0.26 (0.07)	0.01
No Amendment,AWC - Biochar+Compost,IWC	-0.13 (0.07)	0.45
No Amendment,AWC - Compost-only,IWC	-0.08 (0.07)	0.88
No Amendment,AWC - No Amendment,IWC	-0.03 (0.07)	1.00
Biochar+Compost,IWC - Compost-only,IWC	0.05 (0.07)	0.98
Biochar+Compost,IWC - No Amendment,IWC	0.10 (0.07)	0.71
Compost-only,IWC - No Amendment,IWC	0.05 (0.07)	0.98

Chapter 2: The effects of biochar application on native plant communities

Preface:

Biochar is a bio-based soil amendment that can increase soil fertility, forest productivity, and resilience to drought conditions associated with a changing climate. The vast majority of previous biochar research has focused on agricultural applications for production, and no information exists on potential effects on the native plant communities in forested ecosystems. When used in forestry, biochar is often applied into the planting hole with the seedling in small-scale treatments. Discussed here is a replicated study design to explore effects of biochar application rate and torrefaction level on overall plant community development in recently harvested jack pine forests when biochar has been applied as a broadcast topdress amendment. Treatment effects on plant communities were assessed monthly in the first growing season after biochar application. Results indicate that seasonal variation is pronounced in terms of diversity of plant species, in addition to differences between the effects of the different torrefaction levels on the plant communities. Understanding a broader picture of biochar's effects on forest ecosystems will provide important information for forest managers looking to utilize this resource on an operational scale to mitigate predicted impacts due to climate change.

Introduction

Soil amendments have been used for centuries in managed systems as a tool to influence soil productivity (McNeill and Winiwarter 2004). The use and study of soil amendments

to increase productivity and yield has been, and continues to be, broadly studied in agricultural systems around the world. Extensive studies on soil amendments in managed forest systems of the USA are more regional, with greater interest in the southeast and Pacific northwest of the United States and less research in the Great Lakes Region. However, with changing environmental conditions, soil amendments like biochar are being considered as a management tool. Case et al. (2012, 2014) showed significant reductions in European soil CO₂ emissions after application of a biochar amendment. Likewise, Woolf et al. (2010) estimates that 12% of anthropomorphic CO₂ emissions could be mitigated by widespread biochar.

Biochar is a bio-based soil amendment that is created when wood products undergo pyrolysis. This harvest residue is being explored for its potential use in forestry, already being broadly applied in agriculture in the Americas (Lehmann et al. 2006; Sanchez 2019; Leopold and Boyd 1999). Broadcast topdress applications of soil amendments are widely used in the Southeastern United States as a method of forest management (Marx et al. 2005). However, this most commonly applies to fertilizers (inorganic and organic), rather than biomass-based amendments such as biochar. Currently, biochar is most commonly applied in the hole with a planted seedling in small-scale pot and field studies (Kelso 2019; Richard et al. 2017), but there is interest in using it as a broadcast treatment, to minimize application costs and to aid incorporation into the soil. Richard et al. (2017) estimated that applying biochar in this method (applied in seedling hole) doubled planting time and was twenty times more expensive per acre. This is not operationally feasible. The US Forest Service is currently testing different broadcast methods and technologies in the western United States (US) (Page-Dumroese et al. 2016). However, it is relatively

unknown what effect, if any, a broadcast application will have on the native plant communities of the different forest systems. There is also concern of loss pathways when biochar is spread on top and not incorporated into the soil (e.g., via water or wind erosion; Major et al. 2010).

In agricultural systems, biochar is applied as a broadcast and then incorporated into the soils. The largest increases in plant yields has been found with biochar use in tropical agricultural soils (Atkinson et al. 2010; Lehmann and Rondon 2006; Major et al. 2010).

In a short-term study, Schmit et al. (2014) found no immediate effects of a topdress application of biochar in temperate vineyard soils. However, there are limited studies exploring the topdress application impacts--especially in forest systems. It seems reasonable that effects would be dependent on application amount, where effects would increase with increasing rate (depth) of biochar.

Exactly how biochar will influence the system depends both on the soils and the type of biochar product added. In general, biochar has been found to raise the pH of soils it is added to, especially sandy and loamy soils (Glaser et al. 2002; Tryon 1948). This is likely due to concentration of carbonate in the biochar that causes liming to occur, and the low buffering capacity of sandy soils (McElligott et al. 2011; Van Zwaiten et al. 2007). It is a good idea to know the pH thresholds of the species in the system biochar is being applied to, to best determine any positive or detrimental effects to the ecosystem. It is worth noting that the source of the biochar can affect the pH of soils it is applied to, with hardwoods raising the pH higher than conifers, likely due to ash contents (Glaser et al. 2002).

Biochar is not a uniform product; production methods can greatly influence the properties of the biochar that is applied. One factor is its degree of torrefaction, or the temperature and length of time undergoing the pyrolysis process (Shankar Tumuluru et al. 2011). Highly torrefied biochar tends to have finer particles (in addition to higher pH and CEC), while low torrefaction leaves the pieces as larger identifiable woodchips. Studies have found that biochar created with a higher degree of torrefaction tend to be more recalcitrant in the soil (Ahmad et al. 2017; Verhoeff et al. 2011; Luo et al. 2013). These factors could impact vegetative communities by persisting in the soil and altering how much water and nutrients are retained, due to differing water-holding capacities and cation-exchange sites.

In the Lake States, there have been no studies examining effects of applying a topdress amendment of biochar the broad forest vegetation communities. For this reason, we decided to explore the effects that a biochar application may have on the total forest communities, not just individual trees. Previous studies have shown that vigor of aboveground competition has an effect on seedling regeneration and resilience when planted with a biochar amendment, and it is therefore important to know how surrounding vegetation would respond to a widespread application of biochar (Kelso 2019). The goal of this study is to quantify immediate responses of the vegetative plant community during the first growing season after different biochar treatments were applied. This will provide valuable baseline information to natural resource managers in understanding the potential impacts of a broadcast biochar amendment to be able to weigh trade-offs.

Methods

Study Area:

The study took place at the University of Minnesota's Cloquet Forestry Center (CFC), located 225 kilometers north of the Twin Cities, Minnesota in Carlton County. The mission of the CFC is to provide teaching, research, and outreach to the state of Minnesota and the region regarding forest management, forest ecology, and other related fields. The site was a 0.97 hectare jack pine stand on Omega loamy sand soils that was harvested using a clearcut silvicultural system in early October 2017. No residuals were retained and slash was left on site. The study described below was initiated shortly after harvest. The mean annual temperature is 2-3 °C with 70 cm of precipitation, with 165 cm falling as snow (snow water equivalent = 17 cm; NOAAa; NOAAb). The growing season is short, typically running from May to mid-October.

Experimental Design:

This project compared two factors: biochar application rate and degree of torrefaction. Biochar was supplied by the University of Minnesota's Natural Resources Research Institute. Each factor had two levels. Biochar application rate was either 10 t/ha or 20 t/ha; degree of torrefaction was either ~9300 BTU (British thermal units) or ~10000 BTU (Table 2.1, Figures 2-5). Non-treated control areas were also established. Each treatment combination was replicated five times in 1/250th hectare circular plots; the control treatment was replicated 10 times. This resulted in a total of 30 plots (Figure 2.5). For each treatment and control plot, slash was removed within the plot and in treatment plots the designated amount of biochar was evenly spread manually around the plot on top of snow in October 2017.

Table 2.1. Kilograms of biochar applied for each treatment in each 1/250th hectare circle.

	Low torrefaction (~9300 BTU)	High torrefaction (~10000 BTU)
Low application rate	40	10
High application rate	80	20



Figure 2.1. High application rate, low torrefaction



Figure 2.2. Low application rate, low torrefaction



Figure 2.3. High application rate, high torrefaction



Figure 2.4. Low application rate, high torrefaction



Figure 2.5. Aerial view of plots after snowmelt (Photo credit: Andy Jenks)

Vegetation Sampling:

Ten plots of pre-treatment vegetation data (percent cover by species) were collected in September 2017 before the harvest took place. Post-treatment, sampling of plant communities occurred within the treatment plots, but at a smaller internal $1/740^{\text{th}}$ hectare plot to reduce any edge effect. Within each $1/740^{\text{th}}$ circular plot, plot center was established with a bamboo stake and four 1x1 meter plots (using a PVC frame) were marked with pin flags one meter from plot center in each cardinal direction (Figure 2.7). Each month (May 2018-September 2018), percent cover was visually estimated by species to the nearest percent (grasses and ferns were classified by group) within each subplot (Figure 2.6).



Figure 2.6. Vegetation subplot in May 2018 (top) and August (bottom)

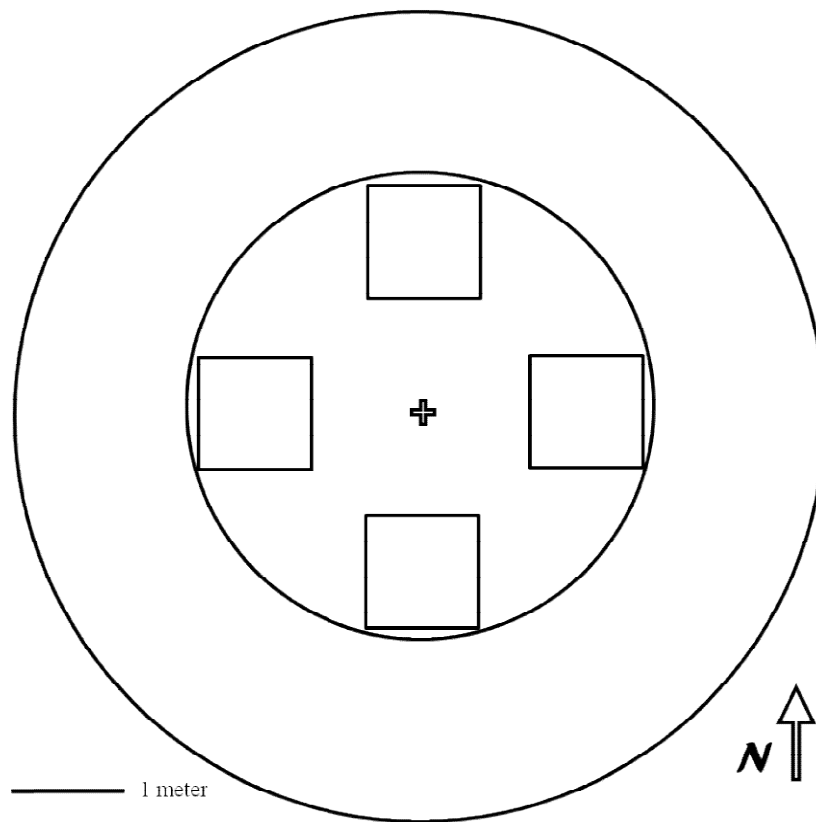


Figure 2.7. Plot and subplot design for vegetation sampling. Larger circle represents 1/250th ha plot, smaller circle represents 1/740th ha plot, cross marks plot center, and squares are the four 1x1m vegetation sampling subplots. Note figure not to scale.

Data analysis:

Summary statistics were calculated for each treatment combination (n=5; averaging all subplots within each plot associated with each treatment combination). Species richness, evenness, and Shannon's diversity index were calculated for all species.

Nonmetric multidimensional scaling (NMS) ordination was used to examine species composition along a gradient of time, biochar application rate, and biochar torrefaction level. NMS was used due to the relaxed assumption of normality and because it does not assume a linear response in species to different gradients (McCune and Grace 2002).

Data were organized at the treatment level, averaging the percent cover of each species for each plot (four subplots per plot, five plots per treatment). Unknown species (present in five plots total) and species present less with less than 0.5% across all plots were deleted from the ordination (ten species total) to reduce the impact of rare species on the ordination (McCune and Grace 2002). No transformations were used for the data. Two ordinations were completed in PC-Ord Version 6 using the slow and thorough autopilot mode with the Sorenson (Bray-Curtis) distance measurement. The first ordination contained percent cover by species for all the months of the study period (May – September) to assess differences across the growing season. A second ordination was run with just the summer months (July – September) to assess differences within the most active part of the growing season. Multivariate analysis of variance (MANOVA) could not be completed due to the unbalanced nature of the study design, which also prevented the Multi-response Permutation Procedure (MRPP) analysis from being blocked by month. Species-area curves were created.

Results

Fifty species were observed across all treatments (Table A2.1, A2.2). Richness, and percent cover of species in plots almost always increased as the season progressed (May through September; Table 2.2; Figure 2.8). Shannon's Diversity typically increased until early/mid-summer, and then decreased (Table 2.2). Evenness was much less consistent. Percent cover of naturally regenerated jack pine seedlings increased through the growing season (Figure 2.9).

Table 2.2. 2018 Percent cover, Species Richness, Shannon's Diversity Index, and Evenness found for each treatment combination.

Month	Rate	Torrefaction	Total % cover	Richness	Shannon's Diversity	Evenness
May	High	Coarse	0.95	11	2.02	0.84
June	High	Coarse	7.95	20	2.16	0.72
July	High	Coarse	26.95	28	2.21	0.66
August	High	Coarse	38.20	26	2.23	0.68
September	High	Coarse	43.95	25	2.24	0.70
May	Low	Coarse	1.58	14	2.16	0.82
June	Low	Coarse	7.85	15	2.31	0.85
July	Low	Coarse	26.65	25	2.45	0.76
August	Low	Coarse	34.80	26	2.27	0.70
September	Low	Coarse	45.05	27	2.14	0.65
May	High	Fine	1.08	10	1.79	0.78
June	High	Fine	8.28	20	2.60	0.87
July	High	Fine	38.95	23	2.20	0.70
August	High	Fine	53.80	26	2.27	0.70
September	High	Fine	53.45	24	2.22	0.70
May	Low	Fine	1.35	16	2.21	0.80
June	Low	Fine	7.33	18	2.51	0.87
July	Low	Fine	27.45	26	2.26	0.69
August	Low	Fine	37.60	25	2.25	0.70
September	Low	Fine	35.85	26	2.20	0.68
May	None	None	1.41	17	2.03	0.72
June	None	None	8.50	24	2.51	0.79
July	None	None	26.46	32	2.62	0.76
August	None	None	38.93	31	2.49	0.72
September	None	None	49.78	32	2.36	0.68

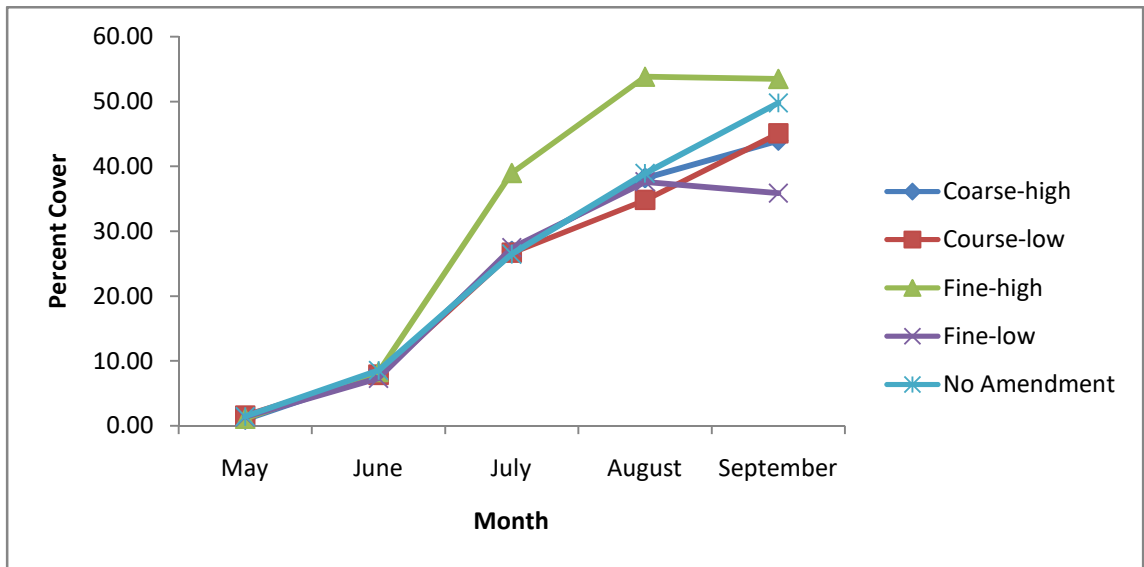


Figure 2.8. Percent cover of all species by month and treatment.

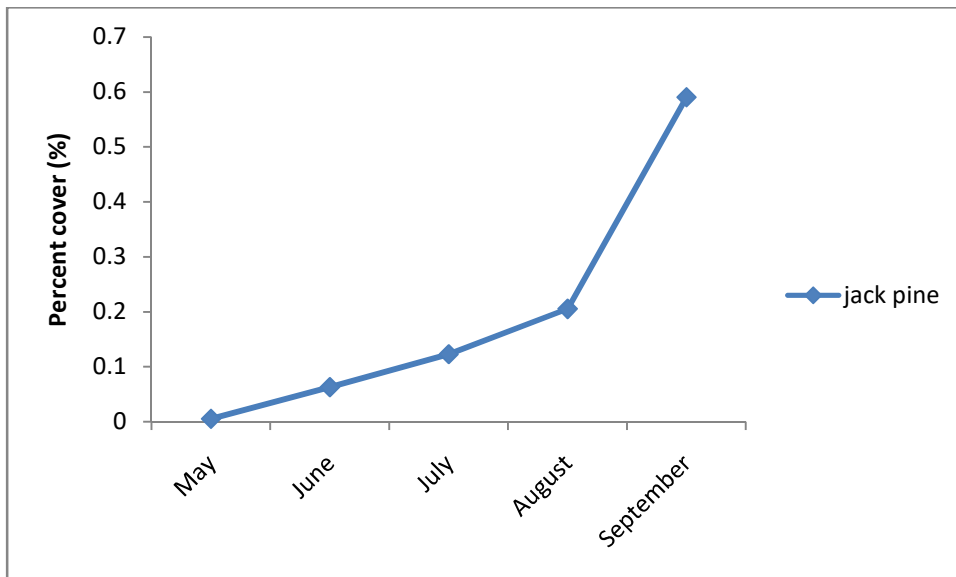


Figure 2.9. Percent cover of naturally regenerated jack pine (*Pinus banksiana* Lamb.) seedlings across all plots through time.

Two ordinations were run to explore underlying gradients in plant community data. The first ordination used the full growing season data set (May – September). This resulted in a final stress of 7.17 and a single axis solution that explained 91% of the variation (Figure 2.10). There was strong grouping by month, with May and June being more different

than July, August, and September (Figure 2.10). Species evenness and richness were strongly correlated with Axis 1 ($r > 0.2$ for both).

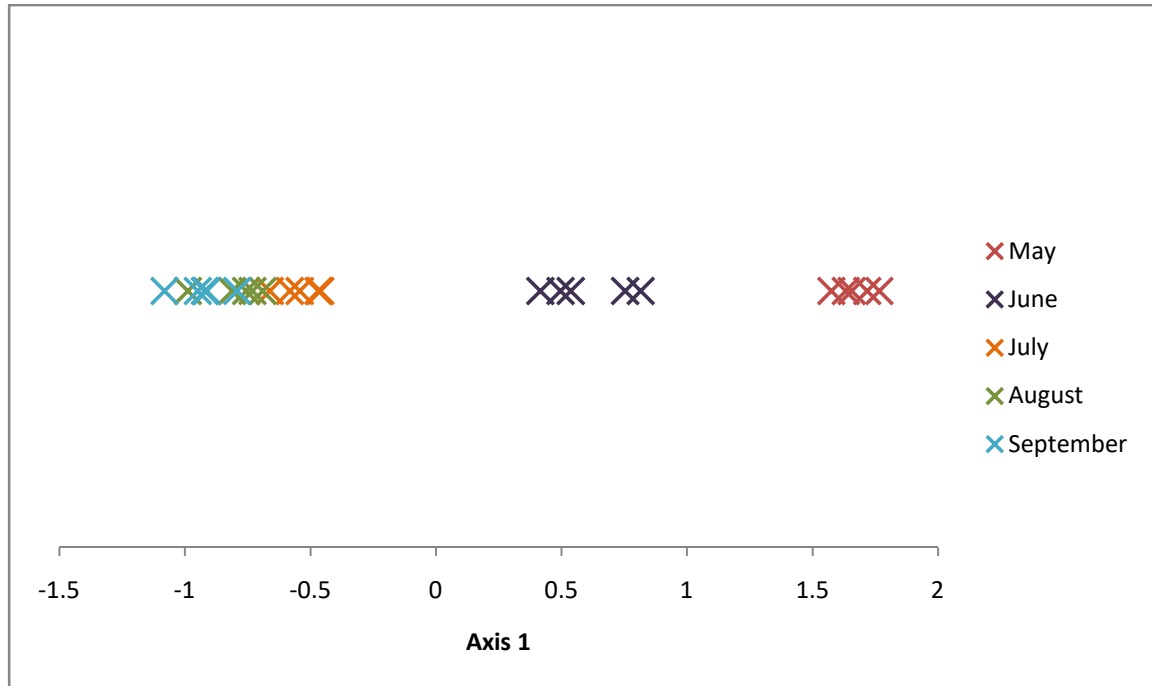


Figure 2.10. Non metric multi dimensional scaling ordination of vegetation observed in each treatment in 2018. Axis 1 explains 91% of the variation. Seasonality effects of a topdress biochar application are shown with different colors.

Since there was strong clustering of data from July, August, and September, the months of May and June were removed from the data set. The second condensed ordination resulted in a three-dimensional figure with a final stress of 4.75 and explaining 94% of the variation. Axis 1 and Axis 2 explained 45% and 24% of the variation, respectively, while Axis 3 explained 25%. There is a strong division among the different biochar material sizes (Figure 2.11). Evenness and Shannon's diversity were strongly associated with Axis 1 ($r > 0.2$ for both). Axis 1 displays a gradient of season development with

more positive values earlier in the summer (July) and more negative values later in the summer (September). Axis 2 relates to the level of torrefaction (Figure 2.11).

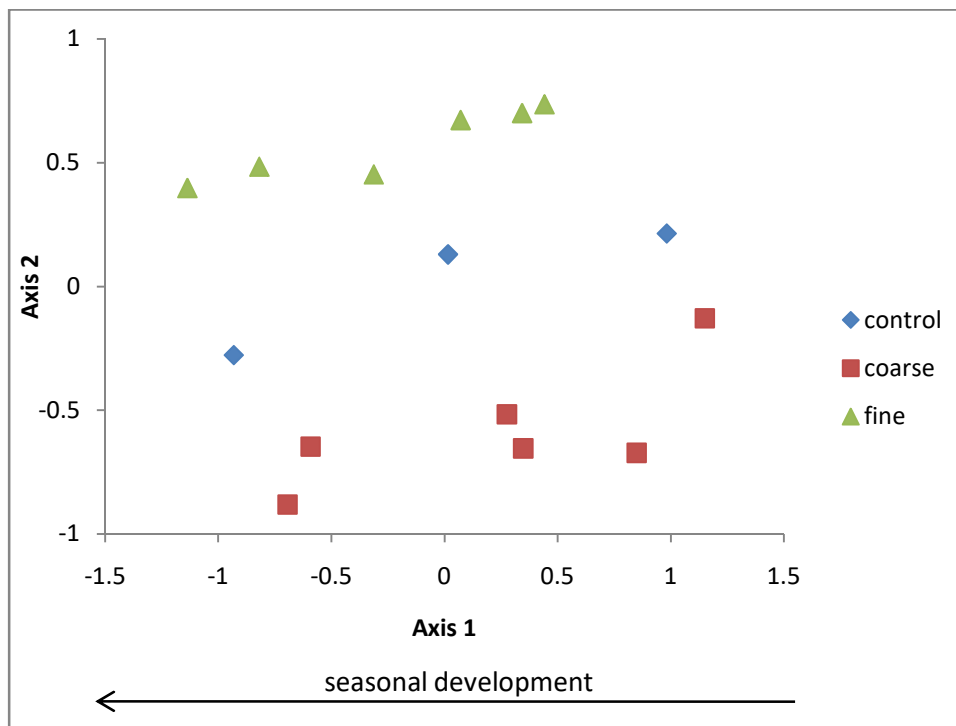


Figure 2.11. Non metric multi dimensional scaling ordination of herbaceous species observed in each treatment in 2018. Axis 1 explains 45% of the variation while Axis 2 explained 24% of the variation. Triangles represent highly torrefied biochar, and squares represent less torrefied biochar. Diamonds represent control plots. Axis 1 from right to left shows effects of seasonality.

For the species-area curves completed, there was a total species richness of 38 species, and the species-area analysis first-order jackknife found 38 species, indicating that the complete species pool was likely sampled. Certain species (such as bed straw and cowwheat) had low occurrence (Table A2.1). Due to the fact that those species are generally more common in mature stands, they are likely artifacts of the previous cover type.

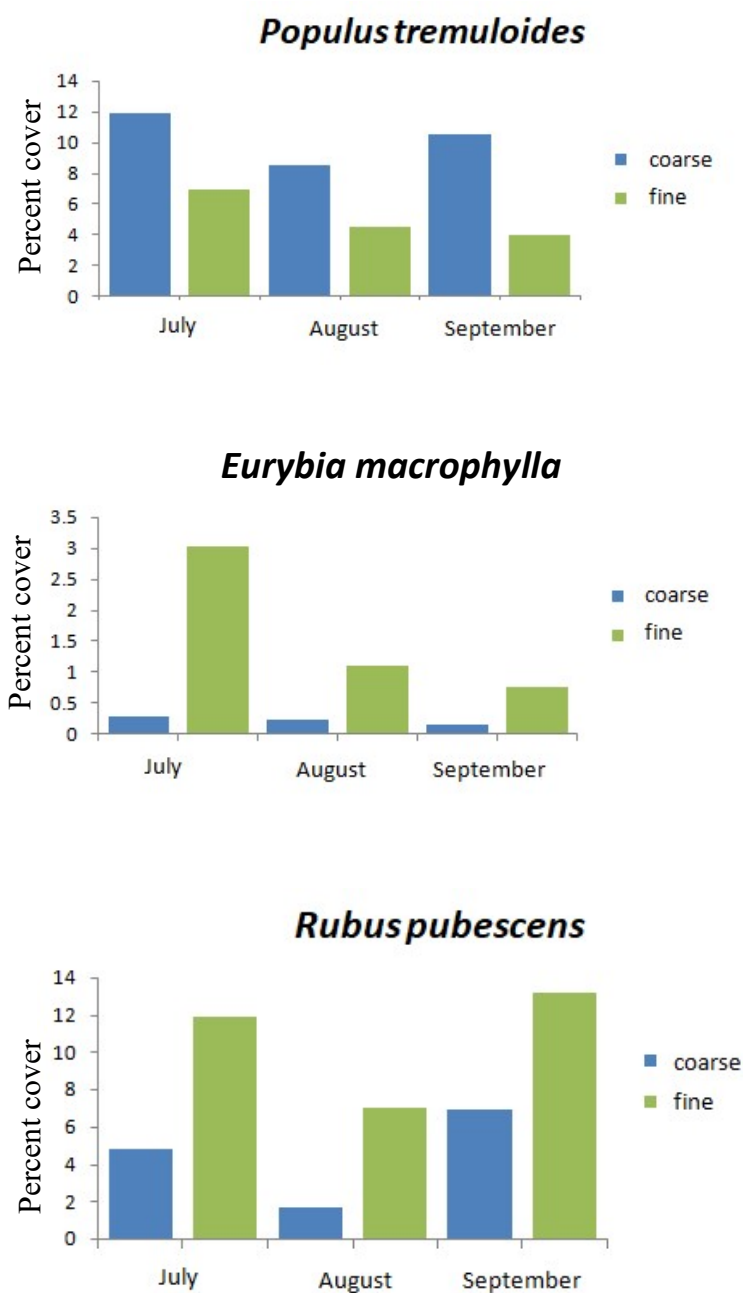


Figure 2.12. Percent cover of *Populus tremuloides* (no stem count available), *Eurybia macrophylla* and *Rubus pubescens* across all coarse (blue) and fine (green) plots during July, August, and September.

Among individual species, there was variability in percent cover response to biochar torrefaction application (Figure 2.12). *Populus tremuloides* showed higher percent cover

in plots with coarse biochar applied, while *Eurybia macrophylla* and *Rubus pubescens* showed higher percent cover in the plots containing the finer biochar (Figure 2.13).

Discussion

With just a single growing season of data, it is not possible to draw long-term conclusions about the effect that different rates and different torrefactions of biochar have on native plant communities when applied as a topdress treatment in a jack pine ecosystem.

However, based on the immediate data collected over the full growing season one year after application, there were not strong differences among the different treatments (Figure 2.11). Jeffery et al. (2011) observed a similar pattern in that there was no correlation found between the different rates of biochar application on crop productivity in a meta-analysis. However, when the data was broken out within the growing season, there were differences in the plant communities between the levels of torrefaction. While the mechanisms causing this are unclear, it could be a result of the differences in hydrophobicity between the coarser and finer particles of biochar and light availability.

Frequent historical surface fires are thought to have decreased the coverage of woody shrubs and other vegetative species (Ahlgren 1960; Nyamai et al. 2014; Van Wagner 1970; Roberts 2004). Surface fires of moderate intensity occurred every 5 to 50 years in these types of systems prior to European settlement (Heinselman 1973). Fire suppression in the region has decreased charcoal additions to the soil, limited tree seedling regeneration, and altered native plant communities (Royo and Carson 2006; Waldrop et al. 1992). Due to the reduction of a natural fire regime in the history of the region, it is important to be aware of how plant communities may have changed as a result.

Challenges may arise when biochar is being applied with topdressing. Major et al. (2010) estimates that 53% of topdressed biochar can be lost from the soil surface through a combination of wind and seepage into the soil, minimizing effects and carbon sequestering properties. Loss of biochar was observed in this study in two ways: blowing during the application process and flowing out of plots during heavy rainfall where there was some topography. The first loss mechanism was affected by the fact that the biochar was spread on top of snow, thus preventing some of the finer particles from blowing away. The second loss pathway could be minimized by incorporating the amendment into the soil, factoring biochar loss into the application rate, or limiting application to relatively flat topography.

While applying a broadcast treatment of biochar is common in agriculture, doing so in a forested system proves difficult due to variable topography and the presence of standing trees. At present, there is no widely-used mechanism to apply such an amendment in forests. However different methods have been developed as a way to spread biochar (Richard et al. 2017; Page-Dumroese et al. 2016). This study used manual spreading, which is likely not feasible to implement on a larger scale.

Short-term effects of a topdress application could include a combination of both shading the soil surface and absorbing sunlight (due to the dark color of the biochar). This would likely be lessened as time progressed, with some biochar being lost in the aforementioned way or by it being incorporated into the soil over time. The latter is hypothesized to drive long-term effects of altering soil properties and resource availability. Certain species appear to be affected differently by differing torrefaction applications within the study period window. Bigleaf aster (*Eurybia macrophylla* Cass.) and dwarf raspberry (*Rubus*

pubescens Raf.) had higher occurrences in the finer biochar treatments than the coarse, while quaking aspen (*Populus tremuloides* Michx.) displayed the opposite effect. This could arise due to the hardness of the seedling, with the woody quaking aspen seedlings more able to persist and outcompete in coarser material because less vigorous seeds may be less able to germinate.

This study aims to set up long-term research plots to examine the effects of biochar as a topdress application on native plant communities. Future research can monitor stem counts of the naturally-regenerated jack pine seedlings that sprouted during the initial year of this study. Knowing how application rates and different torrefactions impact the full forest plant communities can aid managers in assessing the trade-offs of this soil amendment. Plant communities are dynamic; multiple years of data are needed to fully understand the potential impact of biochar treatments. In addition, data could be collected at different times during the year to account for seasonality differences.

However, after one-year of treatment there were limited differences among the different treatments and the overall plant community.

Conclusion and Implications

Understanding the implications that a topdress application of biochar could have on other mechanisms of a forested system (besides the marketable trees) is essential to promoting a healthy, dynamic ecosystem. Setting in place a long-term study with replicated treatments allows researchers to have the ability to observe changes in plant communities over time, and thus be able to better predict and understand the effects of biochar amendments on these communities. It is hoped that the initiation of this study will

inform managers and researchers as they make decisions about biochar application rates, torrefactions, and the future health of the ecosystem.

Appendix

Table A2.1. Species code, scientific name, and common name for all species found across all plots.

Species code	Scientific Name	Common Name
ANQU	<i>Anemone quinquefolia</i> L.	anemone
POTR	<i>Populus tremuloides</i> Michx.	aspen
CLBO	<i>Clintonia borealis</i> Aiton	bead lily
COCO	<i>Corylus cornuta</i> Marshall	beaked hazel
ARUV	<i>Arctostaphylos uva-ursi</i> L.	bearberry
GAAP	<i>Galium aparine</i> L.	bedstraw
AST	<i>Eurybia macrophylla</i> L. Cass.	bigleaf aster
COAR	<i>Convolvulus arvensis</i> L.	bindweed
BEPA	<i>Betula papyrifera</i> Marshall	birch
RUBU	<i>Rubus</i> L.	blackberry
VAAN	<i>Vaccinium angustifolium</i> Aiton	blueberry
COCA	<i>Cornus canadensis</i> L.	bunchberry
MACA	<i>Maianthemum canadense</i> Desf.	canada mayflower
PRUN	<i>Prunus serotina</i> Ehrh.	cherry
TRIF	<i>Trifolium</i> L.	clover
MELA	<i>Melampyrum</i> L.	cowwheat
TARA	<i>Taraxacum officinale</i> F.H. Wigg.	dandelion
RUPU	<i>Rubus pubescens</i> L.	dwarf raspberry
FERN		fern
SOLI	<i>Solidago</i> sp.	goldenrod
COTR	<i>Coptistrifolia</i> L.	goldthread
GRAS		grass
LYDE	<i>Lycopodium dendroideum</i> Michx.	groundpine
CRAT	<i>Crataegus</i> L.	hawthorn
EQUI	<i>Equisetum</i> L.	horsetail
VIRE	<i>Viola renifolia</i> A. Gray	kidney leaved violet
MENT	<i>Mentha</i> L.	mint
SOLA	<i>Solanum</i> L.	nightshade
ANMA	<i>Anaphalis margaritacea</i> L. Benth.	pearly everlasting
ACRU	<i>Acer rubrum</i> L.	red maple
QURU	<i>Quercus rubra</i> L.	red oak
COSE	<i>Cornus sericea</i> L.	red osier dogwood
ARNU	<i>Aralia nudicaulis</i> L.	sarsaparilla
PINU	<i>Pinus</i> sp.	seedling
AMAL	<i>Amelanchier</i> Medik.	serviceberry
SODE	<i>Sorbsdecor</i> Sag.	showy mountain ash

MARA	<i>Maianthemum racemosum</i> L.	solomon's seal
APAN	<i>Apocynum androsaemifolium</i> L.	spreading dogbane
TRBO	<i>Trientalis borealis</i> Raf.	starflower
FRVI	<i>Fragaria virginiana</i> Duchesne	strawberry
COPE	<i>Comptonia peregrina</i> L.	sweetfern
CIRS	<i>Cirsium</i> Mill.	thistle
ROSA	<i>Rosa acicularis</i> Lindl.	wild rose
SALI	<i>Salix</i> L.	willow
GAPR	<i>Gaultheria procumbens</i> L.	wintergreen
ACHI	<i>Achillea</i> L.	yarrow
TRDU	<i>Tragopogon dubius</i> Scop.	yellow salsify
UNK1		unknown 1
UNK2		unknown 2
UNK3		unknown 3

Table A2.2 Species code and percent cover by torrefaction by month across all torrefaction levels.

	Coarse					Fine					Control				
	May	June	July	August	September	May	June	July	August	September	May	June	July	August	September
ANQU	0.08	0.06	0.11	0.38	0.45	0.14	0.28	0.24	0.75	1.20	0.04	0.11	0.29	0.93	0.85
POTR	0.14	1.78	8.58	11.85	10.50	0.11	0.31	4.50	6.98	3.95	0.19	1.83	4.18	6.65	7.40
CLBO	0.21	1.00	0.70	0.20	0.13	0.18	0.25	0.63	0.25	0.18	0.10	0.50	0.33	0.10	0.15
COCO	0.03	0.29	1.09	1.15	0.00	0.00	0.45	1.96	1.80	0.00	0.00	0.20	1.13	1.40	0.00
ARUV	0.00	0.11	0.25	0.30	0.15	0.00	0.26	1.10	3.03	0.75	0.00	0.10	0.75	0.60	0.88
GAAP	0.00	0.00	0.25	0.33	0.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AST	0.00	0.00	0.05	0.10	0.13	0.00	0.00	0.03	0.03	0.13	0.00	0.00	0.25	0.63	0.98
COAR	0.01	0.03	0.08	0.00	0.00	0.03	0.13	0.13	0.13	0.00	0.04	0.18	0.25	0.00	0.00
RUBU	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.13	0.08	0.00	0.00	0.00	0.13	0.00
VAAN	0.05	0.18	0.35	0.48	0.58	0.01	0.10	0.11	0.20	0.30	0.01	0.59	0.20	0.30	0.25
COCA	0.00	0.24	1.15	1.48	2.75	0.00	0.79	2.24	3.35	4.20	0.00	0.39	0.95	0.83	2.05
MACA	0.43	0.88	0.59	0.43	0.28	0.45	0.74	0.64	0.40	0.38	0.63	1.25	0.59	0.40	0.28
PRUN	0.00	0.05	3.13	2.63	0.83	0.00	0.00	2.26	2.88	1.18	0.00	0.00	3.30	2.50	0.30
MELA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.01	0.01	0.00	0.00	0.03
TARA	0.00	0.00	0.08	0.05	0.05	0.00	0.00	0.00	0.03	0.08	0.00	0.03	0.05	0.03	0.48
RASP	0.01	0.28	1.68	4.78	6.88	0.01	0.55	7.01	11.85	13.15	0.01	1.03	3.35	8.58	11.38
FERN	0.06	0.58	4.38	5.20	4.80	0.01	0.38	8.43	7.55	6.78	0.00	0.34	4.93	5.45	5.05
SOLI	0.01	0.00	0.03	0.00	0.00	0.01	0.04	0.01	0.00	0.00	0.05	0.01	0.01	0.00	0.00
GRAS	0.01	0.33	1.38	4.13	11.53	0.05	0.26	0.71	2.98	6.90	0.08	0.30	1.31	4.68	11.83
LYDE	0.05	0.13	0.11	0.10	0.15	0.03	0.00	0.09	0.10	0.08	0.11	0.05	0.16	0.28	0.30
CRAT	0.00	0.00	0.00	0.00	1.20	0.01	0.00	0.00	0.00	1.63	0.00	0.00	0.00	0.00	1.13
VIRE	0.00	0.00	0.14	0.25	0.53	0.04	0.05	0.20	0.35	0.78	0.03	0.00	0.29	0.35	0.23
MENT	0.00	0.03	0.05	0.18	0.03	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.18	0.40	0.20
SOLA	0.00	0.00	0.11	0.23	0.33	0.00	0.00	0.03	0.15	0.20	0.00	0.00	0.55	0.48	0.85
ACRU	0.08	0.23	0.75	0.63	0.53	0.05	0.44	0.66	0.48	0.28	0.04	0.20	1.65	2.10	2.23
ARNU	0.00	0.85	0.40	0.10	0.10	0.00	0.18	0.18	0.13	0.10	0.00	0.39	0.63	0.23	0.15
PINU	0.00	0.03	0.11	0.13	0.48	0.00	0.11	0.18	0.23	0.58	0.03	0.04	0.13	0.33	0.85
AMAL	0.01	0.00	0.03	0.00	0.00	0.01	0.00	0.05	0.00	0.00	0.00	0.00	0.05	0.00	0.00
SODE	0.00	0.01	0.28	0.10	0.00	0.01	0.39	0.19	0.13	0.00	0.01	0.05	0.03	0.05	0.00
MARA	0.03	0.05	0.03	0.00	0.00	0.01	0.06	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
APAN	0.00	0.00	0.03	0.08	0.08	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.03	0.15	0.13
TRBO	0.00	0.76	0.53	0.48	0.35	0.00	1.08	1.00	1.38	0.70	0.00	0.85	0.58	0.80	0.30
FRVI	0.01	0.05	0.23	0.25	0.28	0.03	0.50	0.30	0.10	0.50	0.04	0.01	0.15	0.23	0.35
COPE	0.00	0.00	0.06	0.23	0.45	0.00	0.00	0.08	0.13	0.25	0.00	0.00	0.14	0.18	0.48
CIRS	0.00	0.00	0.00	0.03	0.30	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.08	0.20
ROSA	0.00	0.00	0.00	0.03	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.03	0.05	0.13
SALI	0.00	0.00	0.00	0.13	0.08	0.00	0.00	0.00	0.18	0.05	0.00	0.00	0.00	0.00	0.10

Conclusion

Understanding the effects that a biochar application could have on a temperate forest is extremely valuable. In other systems, it has been found to increase cation exchange capacity, water holding capacity, and nutrient availability (Liang et al. 2005; Basso et al. 2013; Borchard et al. 2014; Steiner et al. 2010; Fischer and Glaser 2012). This is promising as a way of increasing plant productivity and sequestering carbon in the soil. At present, there are few studies that look at the use of biochar in forests, and even fewer long-term studies (Jeffrey et al. 2011). For this reason, it is important to initiate studies that can be monitored over long periods of time, with the intent of discovering how to better manage these systems in the face of a changing climate.

Chapter 1 found that biochar amendments had variable effects on growth and survival of jack pine seedlings, while removing competition significantly increased growth. Wetter-than-average periods of each growing season of the study may have limited the effect of additional irrigation on growth or survival. Chapter 2 found that after a single growing season of having biochar topdressed, there was a difference in diversity between highly torrefied biochar and a more coarse material during the months of July, August, and September. When combining data across all months (May – September) there was little separation among treatments. These are only first year results, however, so longer trends may occur.

Due to the dynamic nature of forest and plant communities in addition to the changing climate, long-term studies are essential for quantifying the change that happens in

ecosystems over time. With these studies in place, there is a framework for future forest biochar research in the Lake States and beyond.

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